

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61F 2/30	A1	(11) International Publication Number: WO 98/53768 (43) International Publication Date: 3 December 1998 (03.12.98)
(21) International Application Number: PCT/US98/11007 (22) International Filing Date: 29 May 1998 (29.05.98) (30) Priority Data: 60/048,320 30 May 1997 (30.05.97) US (71) Applicant (for all designated States except US): OSTEOBIO-LOGICS, INC. [US/US]; Suite 112, 12500 Network, San Antonio, TX 78249 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): SLIVKA, Michael, A. [US/US]; 8635 Timber West, San Antonio, TX 78250 (US). NIEDERAUER, Gabriele, G. [DE/US]; 18810 Redriver Pass, San Antonio, TX 78259 (US). KIESWETTER, Kristine [US/US]; 111 Blue Star, San Antonio, TX 78204 (US). LEATHERBURY, Neil, C. [US/US]; 152 East Pecan Street #803, San Antonio, TX 78205 (US). (74) Agents: WINNER, Ellen, P. et al.; Greenlee, Winner and Sullivan, P.C., Suite 201, 5370 Manhattan Circle, Boulder, CO 80303 (US).		(81) Designated States: AU, BR, CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: FIBER-REINFORCED, POROUS, BIODEGRADABLE IMPLANT DEVICE (57) Abstract A fiber-reinforced, polymeric implant material useful for tissue engineering, and method of making same are provided. The fibers are preferably aligned predominantly parallel to each other, but may also be aligned in a single plane. The implant material comprises a polymeric matrix, preferably a biodegradable matrix, having fibers substantially uniformly distributed therein. In preferred embodiments, porous tissue scaffolds are provided which facilitate regeneration of load-bearing tissues such as articular cartilage and bone. Non-porous fiber-reinforced implant materials are also provided herein useful as permanent implants for load-bearing sites.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakistan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

5 FIBER-REINFORCED, POROUS, BIODEGRADABLE IMPLANT DEVICE

BACKGROUND OF THE INVENTION

The potential repair of articular cartilage in human using porous scaffolds has been described. Mears in U.S. 4,553,272 describes a method for osteochondral repair focusing on the use of starter cells, specific pore sizes in a porous scaffold, and providing a barrier
10 between the two pore sizes. There is no mention of the use of biodegradable scaffolds or the necessity of providing a scaffold that can withstand physiological loads. Hunziker in U.S. 5,206,023 teaches a method for articular cartilage repair involving pretreating the defect area with enzymes to remove proteoglycans, then providing a biodegradable carrier (scaffold) to provide proliferation agents, growth factors, and chemotactic agents.

15 Vert et al. in U.S. 4,279,249 describe a solid biodegradable osteosynthesis device made of a fiber-reinforced composite. The fibrous component is a biodegradable polymer high in glycolide content and the matrix component is high in lactide units. There is no mention of porous devices and the favorable mechanical properties achieved through fiber reinforcement are obtained through typical stacking and layering techniques known in the
20 art. Uniform distribution of fibers throughout the matrix is not disclosed.

The prior art does not appear to teach how to optimize mechanical properties by reinforcing highly porous materials (50%-90% porous). Nijenhuis et al. in Eur. 0,277,678 describe a biodegradable, porous scaffold preferably incorporating biodegradable reinforcing fibers. The scaffold has a bi-porous structure (bimodal pore distribution)
25 made using a combination of solution-precipitation and salt-leaching techniques. Although the fibers are incorporated to "reinforce" the scaffold, no evidence is presented to verify that the mechanical properties are actually improved through such reinforcement and the fibers appear to be randomly aligned.

5 Stone et al. in U.S. Patent 5,306,311 describe a prosthetic, resorbable articular cartilage composed of a dry, porous, volume matrix of randomly or radially oriented, allegedly biocompatible and bioresorbable fibers. Stone's patent speaks mainly of natural polymeric fibers, such as collagen and elastin, which are harvested and purified from xenogenic sources. The fibers are then cross-linked to provide a cohesive scaffold. The
10 ability of the scaffold to support articulating joint forces is not shown.

All publications and patent applications referred to herein are fully incorporated by reference to the extent not inconsistent herewith.

SUMMARY OF THE INVENTION

This invention provides a fiber-reinforced, polymeric implant material useful for
15 tissue engineering, and method of making same. The implant material preferably comprises a polymeric matrix, preferably a biodegradable matrix, having fibers substantially uniformly distributed therein in predominantly parallel orientation as shown in Figures 1 and 2. In preferred embodiments, porous tissue scaffolds are provided which facilitate regeneration of load-bearing tissues such as articular cartilage and bone.

20 The material of this invention is used to prepare porous, fiber-reinforced, biodegradable tissue scaffolds whose fibrous supports are oriented predominantly in a single direction. The scaffold may be implanted into humans or animals to provide support for physiological loads applied parallel to the predominant direction of orientation of the fibers. For example, in an osteochondral site on the femoral condyle, the primary
25 direction of loading is perpendicular to the surface of the cartilage. The oriented fibers act like struts in a bridge support to provide strength and stiffness to the pore walls of the scaffold and provide a characteristic columnar pore architecture especially suitable for cell ingrowth. The orientation of the fibers also causes the mechanical properties of the scaffold to be anisotropic, i.e., the higher strengths provided by the fibers is maximal in

5 the direction parallel to the fibers, thus providing primary support for physiological loads where they are highest.

Orientation of the fibers is achieved through a dissolution-precipitation process combined with a novel kneading and rolling process. Using various amounts of fiber reinforcement, the mechanical properties of the scaffold may be tailored to the host tissue
10 environment for optimal performance.

Alternatively, the polymer used may be non-biodegradable, and/or the implant may be made non-porous (fully dense) for use as a permanent implant, such as a bone plate in locations requiring load bearing.

The fibers are substantially uniformly distributed throughout the polymer matrix,
15 that is the number of fibers present in a selected portion of the matrix which is large enough to contain several macropores should be substantially the same as (within at least about 20% of) the number of fibers present in any other such selected portion of the matrix. "Macropores" are the larger, columnar-shaped voids formed in the process of manufacturing the material as shown in Figures 1 and 2.

20 The invention may be used for a variety of tissue engineering applications, including osteochondral defect repair, partial and full thickness cartilage defect repair, bone graft substitute, bone graft onlay, ligament or tendon augmentation, oral/maxillofacial surgery, and other reconstructive surgery. The invention is particularly useful for, but not limited to, applications where the implant is to be placed in a defect in
25 a load-bearing tissue, i.e., where stresses applied to the implant once placed in the defect are high in one direction compared to relatively perpendicular directions. One example is in an osteochondral or full thickness cartilage defect, where during such activities as normal walking, there are very high compressive stresses perpendicular to the surface of the cartilage, whereas the stresses parallel to the surface are much less. Another example

5 is alveolar ridge augmentation, where primarily one-directional compressive stresses are due to biting or chewing.

The reinforcing fibers may be made of any suitable biodegradable material by methods known to the art or may be commercially available fibers. Polyglycolide (PGA) fibers are currently available from several sources including Albany International,
10 Sherwood Davis & Geck and Genzyme Surgical Products. Fibers from sutures may also be used, e.g., Vicryl® (90:10 poly [glycolide:lactide]) from Ethicon (Johnson & Johnson). They are preferably synthetic fibers, and are preferably of a length short enough not to interfere with processability, e.g., less than about 1 cm. They can be chopped to the desired length from longer fibers, preferably they have a length between about 0.5 mm
15 and about 1.0 cm and more preferably between about 0.5 mm and about 4.5 mm. The fibers preferably have a diameter between about 5 μ m and about 50 μ m, more preferably between about 5 μ m and about 25 μ m.

The reinforcing fibers preferably have mechanical properties that are not substantially compromised when tested in a physiological (aqueous, 37°C) environment.
20 Any biocompatible material can be used to make the fibers. The fibers are preferably insoluble in the solvent used to dissolve the matrix polymer. For articular cartilage repair, the fibers are preferably made from polyglycolide (PGA) or a glycolide-lactide copolymer with a glycolide content above 80%. For bone repair, the fibers may be made of a biodegradable glass such as calcium phosphate or bioactive ceramic. The volume fraction
25 of fibers within the composite scaffold is preferably between about 5% and about 50%, and more preferably between about 10% and about 30%.

The reinforcing fibers used in this invention may alternatively be hollow fibers known to the art. The hollow fibers provide channels to aid in cell and tissue infiltration and can additionally be filled with bioactive agent for delivery to the tissue.

5 Biodegradable polymers or other biodegradable materials known to the art may be used for the biodegradable matrix. Some examples of suitable biodegradable polymers are alpha-polyhydroxy acids, polyglycolide (PGA), poly(L-lactide), poly(D,L-lactide), poly(ϵ -caprolactone), poly(trimethylene carbonate), poly(ethylene oxide) (PEO), poly(β -hydroxybutyrate) (PHB), poly(β -hydroxyvalerate) (PHVA), poly(p-dioxanone) (PDS),
10 poly(ortho esters), tyrosine-derived polycarbonates, polypeptides and copolymers of the above.

The fibers in the implant material of this invention are preferably oriented predominantly parallel to each other, meaning that greater than fifty percent of the total length of the totality of the fibers, and preferably greater than 75%, are oriented in the
15 same direction or within about 20 degrees, more preferably within about 15 degrees, of the same direction. Preferably, at least as great a portion of the total length of the fibers are oriented as close to parallel to each other as depicted in Figure 1, a scanning electron micrograph showing a material of this invention.

The material of this invention is preferably porous. The pores are preferably
20 interconnected to allow cell migration and extracellular matrix continuity. Interconnectivity here is defined as substantial physical continuity of porous space throughout the scaffold. The presence of fibers during the foaming stage of the manufacturing process helps to insure a minimum of closed-cell pores, which in turn, maximizes the number of open cells, a measure of the interconnectivity. It is preferred
25 that pore distribution and size be substantially uniform. Figures 7A and 7B graph pore size distribution in implant materials without fibers in Figure 7A and with fibers in Figure 7B. Significant improvement in uniformity of pore distribution is shown in Figure 7B with its narrow distribution peak compared to that of Figure 7A. Uniformity of pore distribution in a fiber-reinforced material of this invention is defined herein as giving rise
30 to a distribution curve showing significantly more uniformity than the distribution curve of the same material without fibers. Fibers help to insure that pore size is uniform by

5 providing well-distributed nucleation sites during the foaming process. It is preferred that the pores have average linear dimensions (distance between pore walls, also referred to herein as “diameter”) large enough to accommodate ingrowing cells, e.g., at least about 25 μm , and less than about 300 μm , more preferably between about 50 and about 250 μm .

10 The porosity (pore volume) of the scaffold is preferably between about 50% and about 80%, and more preferably between about 60% and about 70%. Ideally, the scaffold should be sufficiently porous to facilitate tissue regeneration but not so porous as to compromise its mechanical integrity. The oriented fiber-reinforced material has a characteristic columnar architecture which is “biomimetic” of the columnar cell orientation of chondrocytes in articular cartilage.

15 The porous material of this invention may be used as a tissue scaffold *in vivo* or *in vitro*. That is, the material acts as a scaffold (framework) providing support and spaces for ingrowth of cells either after it has been placed within a tissue defect in the patient's body, or alternatively, the scaffold material of this invention may be preseeded with autologous or allogenic cells or cell-containing media before implantation. By
20 adding cells to the scaffold *ex vivo* before implantation, the formation of the desired tissue or organ type can be accelerated. For example, addition of bone marrow to the implant accelerates the formation of bone throughout the scaffold due the presence of osteoprogenitor and angiogenic cells. Addition of hepatocytes to a scaffold material has been reported to form liver tissue; similarly, addition of chondrocytes has been reported
25 to form cartilage.

Cells can be pre-treated with growth and differentiation factors to induce proliferation or a desired phenotype.

5 The implants of this invention may be used to deliver bioactive agents such as growth factors, antibiotics, hormones, steroids, anti-inflammatory agents and anesthetics in timed manner, such as in a timed burst or a controlled-release pattern.

10 The implant material of this invention may be used as one phase of a multiphase implant, e.g. as described in U.S. Patent No. 5,607,474, incorporated herein by reference to the extent not inconsistent herewith. Preferably, the implant material of this invention has mechanical properties similar or identical to those of the tissue into which the implant is to be placed, controlled by the amount and type of fibers used. The effect of fiber content on mechanical properties is shown in Figures 3-6.

15 Methods of making implant materials are also provided herein. A method for making a fiber-reinforced, porous, biodegradable tissue scaffold implant material comprising fibers aligned predominantly in one direction comprises:

- a) dissolving a biodegradable polymer in a suitable organic solvent to form a solution;
 - b) dispersing the fibers in a suitable non-solvent for the polymer to form a suspension.
 - c) precipitating the polymer mixed with fibers as a coherent mass from solution by mixing the suspension and solution;
 - d) kneading and rolling the coherent mass of fibers and polymer to orient the fibers predominantly parallel to each other; and
 - e) applying heat and vacuum pressure to the mass to foam and cure it.
- 20
- 25

5 Methods for the preparation of precipitated polymers are well-known to the art. In general, the process comprises mixing a dried polymer mix with a solvent, preferably acetone, precipitating the polymer mass from solution with a non-solvent, e.g. ethanol, methanol, ether or water, extracting solvent and precipitating agent from the mass until
10 the composition to the desired shape and stiffness. Kneading and rolling may be performed as described in PCT Publication WO 97/13533, incorporated herein by reference to the extent not inconsistent herewith. The kneading may be done by hand or machine and should be continued until the fibers are predominantly aligned substantially parallel to each other, e.g. until the mass becomes difficult to work. Kneading and rolling
15 should be stopped just short of the point where the mass becomes too hard to press into the mold. If it is desired to align the fibers in a single plane, the mass should be rolled out for placement in a flat, shallow mold. If it is additionally desired to align the fibers in a single direction, the mass should additionally be stretched in the desired direction of alignment. Placement in a cylindrical mold is preferred when fiber alignment in a single
20 direction is desired, with molds having a length to diameter ratio of about 1 to 10 being preferred. Increasing degrees of parallel fiber orientation can be achieved (up to a limit) by increasing the length to diameter ratio. Curing and foaming the polymer in the mold to form a porous implant may then be done.

25 After the fibers have been dispersed in the porous scaffold, the scaffold may optionally be pressed, e.g., compression molded, preferably at elevated temperatures until all the pores have been collapsed into a fully dense (non-porous) composite. This approach is especially effective in creating a fiber reinforced composite in which fibers are evenly dispersed and results in good interfacial bonding between the fibers and the matrix.

30 The temperatures required for pressing are as known to the art, those at which the material softens sufficiently to allow intermingling of the polymer chains and

5 collapse of the pores under the pressure being applied. The time and temperature used for compression molding should be sufficient to ensure that no significant residual stresses are present (i.e., the material does not expand when the pressure is released. Using an amorphous PGA (75:25), for example, a temperature of about 100°C is used. For semi-crystallizing polymers such as L-PLA, a temperature of at least about 180°C is
10 required.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a scanning electron micrograph of a porous, fiber-reinforced biodegradable scaffold implant material showing predominantly parallel orientation of fibers and a characteristic columnar pore architecture.

15 Figures 2A and 2B show two cross-sections of a porous, fiber-reinforced implant viewed using a polarized light microscope (A) end-on, viewed parallel to the predominant orientation of the fibers and (B) side, viewed perpendicular to the predominant orientation of the fibers. The fibers appear bright due to their birefringent properties.

20 Figure 3 is a composite plot of Young's modulus and porosity of implant materials of this invention composed of PGA fibers reinforcing 75:25 polylactide:glycolide. The white bars indicate porosity. The black bars indicate Young's modulus. The term "plate" designates materials made by a process in which fibers are predominantly aligned in a single plane. The term "cyl" designates materials made by a process in which fibers are predominantly aligned in a single direction. The percent designation indicates percentage
25 of fibers present. When this percent designation is absent, the materials were made by the process indicated by "plate" or "cyl" with no fibers present.

Figure 4 is a plot of the yield stress of the implant materials shown in Figure 3.

5 Figure 5 is a composite plot of the Young's modulus and porosity of implant materials of this invention made with 75:25 polylactide:glycolide and varying percentages of fiber with figure designations as set forth for Figure 3 above.

Figure 6 is a plot of the yield stress of the implant materials shown in Figure 5.

10 Figures 7A and 7B show pore size distribution plots, constructed using mercury porosimetry analysis on porous implants made (A) without fiber reinforcement and (B) with fiber reinforcement. The narrow peak in (B) shows that the fiber-reinforced implant has a more uniform pore size distribution than that without fiber reinforcement (A).

DETAILED DESCRIPTION

15 A fiber-reinforced implant material is provided in which the fibers are aligned predominantly parallel to each other. Alternatively, fibers may be aligned in a single plane. The implant material is preferably porous as shown in Figures 1 and 2. Alternatively, it may be fully dense (non-porous). Porous implants are useful as tissue scaffolds for tissue ingrowth, and are preferably biodegradable. Non-porous implants may be non-biodegradable and used as permanent implants.

20 The implant described herein may be used to deliver bioactive agents such as growth factors, antibiotics, hormones, steroids, anti-inflammatory agents, and anesthetics. Such agents can be adsorbed onto the implant after manufacture or just prior to implantation, delivered in a controlled manner by secondary vehicles incorporated into the implant, or incorporated into the matrix directly. For an immediate bolus release, the
25 implant can be coated by simply applying the agent to the implant in an appropriate vehicle. One example would be dripping a solution containing the agent over the surface of the implant or allowing the implant to soak in the solution for a short time. Upon

5 implantation, a growth factor release pattern provided by this type of treated scaffold can induce an initial rapid proliferation of cells to fill the space within the porous scaffold.

 Encapsulation methods for such bioactive agents are generally well described in the art, and these methods can be used for preparing materials for incorporation into the present implant device. Hollow fibers known to the art can be used as reinforcing fibers
10 herein and filled with various bioactive agents as taught by Schakenraad et al. (1988), "Biodegradable hollow fibers for the controlled release of drugs," *Biomaterials* 9:116-120; and Greidanus et al., U.S. Patent 4,965,128, both of which are incorporated herein by reference to the extent not inconsistent herewith. The hollow fibers can additionally aid in providing channels for cell/tissue infiltration. This serves the dual purpose of
15 providing the requisite mechanical properties to the implant and delivering the bioactive agent.

 A variety of polymer compositions can be employed to give varying mass degradation rates as well as varying bioactive agent delivery rates. The bioactive agent can be delivered in a controlled manner to achieve a desired period of release or release
20 at a specific point in time. The bioactive agent can be incorporated into the matrix directly during manufacture, as is described by Athanasiou et al. in U.S. patent application 08/452,796, "Continuous Release Polymeric Implant Carrier," which achieves a continuous release of the factor over a specified time. Alternatively, encapsulation via one of the many techniques known in the art can be used. By
25 appropriate selection of polymer and encapsulation method, release can be achieved over a very broad range of times, from virtually immediate release to delayed release of up to 6 months. A delayed release could be desirable for delivering appropriate factors at different times during the healing process. For example, in a cartilage repair model, a proliferation factor such as PDGF-BB can be delivered quickly to the defect site and then,
30 when a sufficient population of cells are present in the scaffold, a differentiation factor such as TGF- β to induce the formation of type II collagen can be delivered.

5 As described above, the implant may be preseeded with autologous or allogenic cells prior to implantation. There are a number of techniques for adding cells to scaffolds. In the present invention, cells can be loaded by immersing the implant materials in a suspension of cells and gently agitating for about 2 hours. Cells infiltrate the scaffold, adhere to the polymer matrix, and go on to proliferate and differentiate. Alternatively,
10 a vacuum-loading method can be used, in which the implant is immersed in a suspension of cells and a gentle vacuum (about 300 mm Hg) is slowly applied. It is important to apply vacuum slowly so that excessive shear forces and cell lysis are avoided within the implant. Yet another cell-loading technique is to infiltrate the scaffold material by centrifugation. The implant is fixed in the bottom of a small centrifuge or microfuge tube
15 and a suspension of cells added. The implant/ cell combination is then spun at 200-1000 x G for 5 to 15 minutes. Excess solution is decanted and the loaded implant may be removed for immediate implantation or incubated for a short-term culture period before implantation into the patient. Cells used for implant seeding can be acquired in a number of ways. They can be isolated from autograft tissue, cultured and expanded from
20 autograft tissue, or derived from donor allograft tissues.

When the fiber-reinforced scaffold is used for articular cartilage regeneration, the fiber and matrix combination is preferably selected such that the mechanical properties of the composite scaffold are tailored to optimal performance for use in an articular cartilage environment. Optimal performance may be determined based on the mechanical
25 properties of the scaffold tested under physiological conditions immediately after wetting and after being immersed *in vitro* in physiological solution, i.e., phosphate buffered saline, synovial fluid, etc., or *in vivo* for critical time periods. For articular cartilage repair, it is preferred that, after 2 weeks of immersion *in vitro*, the compressive Young's modulus of the scaffold be within about 50% of that of articular cartilage tested under
30 identical simulated physiological conditions and testing modality. Simulated physiological conditions include at least an aqueous environment maintained at 37°C. It is preferred that the yield strength of the scaffold be at least about 1 MPa, more

5 preferably at least about 2 MPa, after two weeks of immersion and testing under said conditions. Such a yield strength gives the scaffold sufficient mechanical integrity to withstand the stresses experienced during normal human gait. Furthermore, it is preferred that the scaffold maintain at least 50% of its yield strength, tested under said conditions, after two weeks of immersion time.

10 The mass degradation rate of the scaffold is also a property which may be tailored for optimal performance. As known to the art, mass degradation rate may be controlled by type of biodegradable polymer, molecular weight, and degree of crystallinity (for semi-crystalline polymers). For repair of articular cartilage, it is preferred that at least about 90% of the scaffold be resorbed by between about eight weeks and about 26 weeks
15 after implantation. For bone repair, it is preferred that at least about 90% of the scaffold be resorbed between about ten weeks and about 16 weeks, but by no more than about 26 weeks after implantation.

To make the fiber-reinforced scaffolds of this invention, the reinforcing fibers and matrix polymer are selected as described above. The matrix polymer is dissolved in an
20 appropriate amount of solvent such that the viscosity of the completely dissolved solution is roughly similar to that of motor oil (50-500 centipoise). The reinforcing fibers are immersed in a non-solvent of the polymer. The fibers are left to soak in the non-solvent for at least 15 minutes so that the non-solvent will wet the fibers as much as possible. After letting the fibers soak, they are mixed or blended into a slurry with the non-solvent
25 to disperse the fibers and remove any clumping. It is preferred that extra non-solvent be added to the slurry during blending or mixing. Blending or mixing should be carried out until there are no visible dense clumps of fibers within the slurry. After blending or mixing, the non-solvent is decanted so that only the amount of non-solvent needed for precipitation of the polymer (at least about 90% of the dissolved polymer is desirably
30 precipitated) remains.

5 Once the matrix polymer is completely dissolved and the fibers are well dispersed in the non-solvent, the two are added together and stirred, preferably with a non-stick utensil. Stirring should be continued until an opaque, cohesive gel has precipitated and can be lifted out onto a non-stick surface. The gel is then kneaded to disperse the fibers uniformly within the gel. Uniform dispersion can be assessed by visual observation of the gel when flattened. The gel is kneaded until the fibers are uniformly dispersed and most of the supernatant solvent and non-solvent has been squeezed out. A non-stick roller is then used to flatten the gel on the non-stick surface.

15 An initial vacuum foaming step is then performed to remove more of the residual solvent and non-solvent and to initiate the nucleation of air bubbles in the gel. A vacuum pressure of about 700 mm Hg (60 mm absolute) is preferably pulled on the gel for about 1 to 2 minutes. Following this vacuum step, the gel is fed through rollers separated by a gap of preferably about 0.25 to 0.75 mm for a sufficient number of times that the gel dries further and becomes tough to knead.

20 At this point, the gel is shaped according to the type of mold being used; the type of mold to use depends on the type of orientation of the fibers desired. If one desires the fibers to be substantially aligned in one direction, the mold used should have a cylindrical shape with a length to diameter ratio of at least about 5:1. The gel is rolled to the length of the cylindrical mold and then placed within the mold. If one desires the fibers to be substantially aligned in one plane, the mold should be a parallelepiped having a maximum ratio of about 1:5 between the smallest dimension and each of the other two dimensions, or cylindrical with a length to diameter ratio of at most 1:5. The gel is flattened using a non-stick rolling pin to fill the largest two dimensions of the parallelepiped mold or the circumference of the cylindrical mold.

25 Following kneading, the rolled or flattened gel is placed into its mold, and the mold is closed and placed into a vacuum oven. In this second vacuum foaming step, the

5 vacuum pressure should reach at least about 700 mm Hg and the temperature should be set sufficiently high as to remove residual solvent from the gel but not so high as to cause significant coalescence of pores during curing, e.g. about 65°C. During this step, the gel foams and expands to the walls of the mold and enough of the residual solvent and non-solvent is extracted so the porous composite can be removed from the mold without
10 deformation of the composite. The length of time for the second vacuum step is typically about one day, but two or three days may be necessary for successful results. Following removal of the composite from the mold, a sample of the composite should be analyzed for residual solvent and non-solvent levels. Subsequent drying of the composite outside of the mold may be necessary, as the residual levels should be no more than 100 ppm.
15 The composite may then be cut to its desired shape and orientation depending on the type of application for which it will be used.

Scaffolds of the present invention can be used in the treatment of partial thickness, full thickness and osteochondral cartilage defects. A partial thickness defect is defined as one in which the depth of the defect is only a portion of the entire thickness of the
20 cartilage layer. A full thickness defect is defined as one in which the defect penetrates through the entire thickness of cartilage, but does not extend into the underlying subchondral bone. An osteochondral defect penetrates both the cartilage and the bone. In some instances, it may be desirable to treat a cartilage defect with a multiphase implant which protrudes into the subchondral bone. A multiphase implant is defined as an
25 implant device which consists of one or more layers. This device may include a solid film, a cartilage phase, a bone phase, etc.

To treat a full thickness defect, the invention is preferably used as a single phase implant thick enough to fill the defect. For example for humans, an implant 8 mm in diameter with a thickness of 2.0 mm might be a typical size. The implant will preferably
30 have a biodegradable film covering the articulating surface of the implant to allow for a smooth surface during articulation; however, this film is not a requirement. The implant

5 is then preferably loaded with a suspension of autologous cells isolated from an articular, costochondral, perichondrial, or any other hyaline cartilage donor site. After isolation of the cells from the tissue, they can be expanded in culture, pre-treated with growth factors, hormones, or other biologically active molecules, or they can be used neat without further treatment. Once the cell suspension is prepared, the cells can be loaded onto the implant.

10 To prepare the implant, the sterile scaffold is added to the prepared cell suspension, using just enough media for the cell suspension to cover the implant. The cell/scaffold combination is then shaken gently in an orbital shaker for 1-2 hours to infiltrate the porous material with cells. The implant, wetted with media, is then removed and transferred to the surgical site, maintaining the implant at 37°C to assure the viability
15 of the cells. Alternatively, the scaffold/cell combination can be maintained under culture conditions for an extended period of time to allow the cells to attach or even to begin to proliferate and form extracellular matrix.

To treat a full thickness defect on the femoral condyle, for example, the surgeon preferably exposes the femoral condyle of the affected knee and uses a circular boring
20 tool to cut a "ring" around the damaged site. Using a curette or other appropriate tool, the surgeon then removes all of the damaged cartilage. Once the defect site is prepared, a small amount of fibrin glue or other bio-compatible tissue adhesive is applied to the bottom of the defect site. In selecting the adhesive, it is important to ensure compatibility with the implant materials. The prepared implant is then press fit into the defect site until
25 the surface of the implant is flush with the surface of the cartilage, using the tissue adhesive to secure the implant in place. Alternatively, the prepared implant can be mechanically attached into the defect site using sutures, anchors, rivets, microscrews, tacks, etc., all of which can be made from a biodegradable material. The site is then closed routinely and the appropriate post-surgical therapy prescribed. Continuous passive
30 motion, electrical stimulation, or other treatment can be used as deemed appropriate; however, weight bearing on the treated leg should be avoided or minimized for at least

5 the first 4 to 6 weeks. Repair should be nearly complete within 12 weeks, with complete recovery expected in 4 to 6 months. Optimal post-operative treatment/rehabilitation regime can be determined by the surgeon/physician.

Example 1

Method of Producing a 10% Chopped PGA Fiber-Reinforced, Porous, 10 Biodegradable Implant

To manufacture a 70 volume % porous, 10 volume % chopped PGA fiber-reinforced, 75:25 poly(D,L-lactide-co-glycolide) (D,L-PLG) wafer, the following procedure was used. First, 1.38 g of 75:25 D,L-PLG (Mw = 95,000 Da, intrinsic viscosity = 0.76) was dissolved in a Teflon beaker using 6.2 mL of acetone. Next,
15 0.153 g of PGA fibers with a diameter of approximately 15 μ m, chopped to an average length of about 2.6 mm, were placed in a scintillation vial. Then, 6.2 mL of ethanol was added to the vial with the fibers. The level of fluid in the vial was marked with a permanent marker. Next, the fibers and ethanol were transferred to a Waring blender with an additional 20 mL of ethanol. The mixture was blended on setting 2 for a
20 period of 1 minute. The fiber and ethanol mixture was then added back into the scintillation vial, and the ethanol was decanted until the height of the mixture decreased to the marker.

After the 75:25 D,L-PLG was completely dissolved in the acetone, the fiber and ethanol mixture was poured into the polymer solution and mixed with a Teflon
25 policeman. The solution was mixed until a cohesive gel of precipitated polymer and fiber mass had formed. The gel was then removed from the supernatant acetone and ethanol. Next, the gel was kneaded by hand, taking particular care to manually disperse the fibers within the composite gel. When the gel became somewhat dry and the fibers appeared well distributed upon macroscopic observation, it was processed
30 nine times through rollers separated by a distance of about 0.25 mm. Next, the gel was rolled by hand into a cylindrical shape with a length equal to the length of the

5 mold, which was 5 cm. The gel was then placed in the cylindrical polypropylene mold with a 1 cm diameter and a 5 cm length. During this rolling process, the chopped fibers in the gel became preferentially oriented in the longitudinal direction of the cylinder. The mold was then placed in a vacuum oven preheated to a temperature of 65 °C and a vacuum was drawn to at least 700 mmHg. After one day
10 in the mold in the vacuum oven, the gel had foamed to the size of the mold and had dried to a stiff, structural porous composite. It was then put back in the vacuum oven, open, to remove residual acetone and ethanol, for at least three days.

After the dried, porous composites were removed from the vacuum oven and before any other characterization and use, the residual amount of acetone and ethanol
15 from a portion of the composite was measured using gas chromatography. Subsequent reinsertion into the vacuum oven at 65 °C was done until the residual solvent levels fell below 100 ppm.

To process the cylindrical wafer into the desired shapes and sizes, the following procedures were used. The cylindrical wafer was mounted on a Buehler Isomet 1000
20 Saw and cut transversely to the desired cylindrical height using a diamond-coated saw blade with water as a coolant. The chopped cylinders were then sized to the desired diameter using a hollow coring tool mounted on a drill press.

Example 2

Mechanical Testing to Show the Influence of Fiber Orientation on the 25 Mechanical Properties of Porous, Biodegradable Implants

To make porous wafers with reinforcing fibers oriented preferentially in one direction, the manufacturing method described in the detailed description of the preferred embodiments and Example 1 was used. The addition “cyl” is attached to those implants made using this method, to indicate the cylindrical molds that were
30 used. To make implants with reinforcing fibers oriented in two directions, a plate-like

5 mold was used with dimensions 6 cm x 6 cm x 3 mm thick and the gel was flattened to fit the sides of the mold before the final vacuum step. The addition “flat” is attached to the implants made using this method, and indicates the flat, plate-like form of the cured wafer.

10 The matrix polymer used was a random copolymer of 75:25 poly(D,L-lactide-co-glycolide) and the fibers were poly(glycolide) with a diameter of about 15 μ m and an average length of about 2.5 mm. The dimensions of the samples used for mechanical testing were 6 mm diameter by 3 mm height, obtained using the methods described in Example 1.

15 The method used to measure the mechanical properties of the cylindrical wafers was an unconfined parallel plate compression test. The apparatus consisted of a calibrated Instron Model 5542 Load Frame and Instron 500N capacity tension-compression load cell equipped with an environmental bath, loaded with deionized water and capable of maintaining a temperature of 37 °C. Cylindrical samples mentioned above were preloaded with deionized water by pulling a vacuum on the
20 immersed samples and were preconditioned in deionized water for one hour prior to testing in an oven maintained at 37 °C. Before testing, each sample was removed from the oven, measured using calipers to determine thickness and diameter, and immediately immersed in the bath between unconfined parallel compression platens. The samples were then compressed at a strain rate of 10% per minute and the stress
25 versus strain data obtained from the test were collected and analyzed according to ASTM D 1621-94, “Standard Test Method for Compressive Properties of Rigid Cellular Plastics.”

30 Samples were tested either parallel (||) or perpendicular (\perp) to the preferred orientation of the fibers. Implants without fiber reinforcement were made and tested in the same configurations as those with fiber reinforcement. The parallel or

perpendicular description for these implants indicates that samples were made and tested in the same configuration as the corresponding fiber-reinforced implant. Five groups of samples were manufactured and tested according to the following descriptions:

1. 75:25 D,L-PLG (w/o fibers), flat, \perp
2. 75:25 D,L-PLG (w/o fibers), cyl, \parallel
3. 10% PGA fibers/90% 75:25 D,L-PLG, flat, \perp
4. 10% PGA fibers/90% 75:25 D,L-PLG, cyl, \parallel
5. 10% PGA fibers/90% 75:25 D,L-PLG, cyl, \perp

From the stress versus strain data and postprocessing analysis, the Young's modulus and yield stress of the samples were compared. This was correlated with the porosity of the samples, calculated from the dry mass, thickness, and diameter of the cylindrical samples and density of the polymers used. A composite plot of the Young's modulus and porosity of each of the implants is given in Figure 3. A plot of the yield stress of each of the implants is given in Figure 4.

Figure 3 shows that a porosity of 60% to 70% was maintained for all implants tested. Figures 3 and 4 show, first of all, that without fiber reinforcement, the manufacturing method did not influence the Young's modulus of the implants, and only slightly changed the yield stress. Secondly, Figures 3 and 4 show that preferential orientation of the fibers in the reinforced implants caused a much larger increase in the Young's modulus and yield strength when tested parallel to the direction of preferential orientation. Furthermore, when the implants were made in the plate-like mold, addition of reinforcing fibers only mildly increased the Young's modulus and did not significantly change the yield strength when tested perpendicular to the preferential orientation of the fibers. Figures 3 and 4 also show that the fiber-reinforced implants made in the cylindrical mold have anisotropic mechanical properties; the Young's modulus and yield strength were

5 much greater when tested parallel to the direction of preferential orientation of the reinforcing fibers.

Example 3

Mechanical Testing to Show the Influence of Increasing Levels of Chopped PGA Fiber-Reinforcement on Porous, Biodegradable Implants

10 Implants were made according to the method described in the detailed description of embodiments and in Example 1. The matrix polymer used was a random copolymer of 75:25 poly(D,L-lactide-co-glycolide) and the fibers were poly(glycolide) with a diameter of about 15 μm and an average length of about 2.5 mm. Implants were made with fiber volume fractions of 0%, 5%, 10%, 15%, and
15 20%. Cylindrical samples of about 6 mm diameter and 3 mm length were prepared according to the description in Example 1. The parallel plate compression test method described in Example 2 was used to measure the mechanical properties of the cylindrical wafers. The wafers were tested parallel to the direction of preferential orientation of the fibers.

20 From the stress versus strain data and postprocessing analysis, the Young's modulus and yield stress of the samples were compared. This was correlated with the porosity of the samples, calculated from the dry mass, thickness, and diameter of the cylindrical samples and density of the polymers used. A composite plot of the Young's modulus and porosity of each of the implants is given in Figure 5. A
25 plot of the yield stress of each of the implants is given in Figure 6.

Figure 5 shows that the Young's modulus increased linearly with increasing addition of reinforcing fibers. Figure 5 also shows that the porosity of the implants was maintained between 60% and 70%. Figure 6 shows that the yield strength of
30 the implants also increased with increasing fiber reinforcement.

5

Example 4**Compression Molding of Chopped PGA Fiber Reinforced Composite Material**

10

Fiber-reinforced material was made according to Example 1. This material was then placed into a stainless steel mold, which was then placed between two heated platens in a Carver Laboratory Press. The platens were heated to about 100°C and a pressure of 10,000 lbs was applied. After about two minutes, the mold was cooled to room temperature and the pressed composite material was removed. The final material was fully dense with a uniform distribution of fibers evidence when held up to light.

15

20

Those of ordinary skill in the art will appreciate that alternative techniques, procedures, methods and reagents other than those specifically described in the foregoing examples can be readily employed or substituted to achieve the objects of the present invention, i.e., polymeric fiber-reinforced compositions with fibers aligned in predominantly parallel or planar alignment, and methods for making same. Alternative, but functionally equivalent, compositions and methods will be readily apparent to those of ordinary skill in the art without expense of undue experimentation. All such alternatives, variations and equivalents are to be considered to be encompassed within the spirit and scope of the present invention.

5 CLAIMS:

1. A fiber-reinforced polymeric implant material comprising a polymeric matrix having fibers of a length no greater than about 1 cm substantially uniformly distributed therein in predominantly parallel orientation.
- 10 2. A fiber-reinforced polymeric implant material comprising a biodegradable matrix adapted to be a tissue scaffold implant for placement in a defect in a load-bearing tissue, said implant being porous, and said fibers being aligned predominantly parallel to the primary direction of loading on such implant after placement in said load-bearing tissue.
- 15 3. The implant of claim 1 or claim 2 wherein the fibers are composed of a biodegradable material.
4. The implant of claim 1 or claim 2 used to deliver bioactive agents.
5. The implant of claim 1 or claim 2 seeded with cells prior to implantation.
6. A multi-phase implant comprising as at least one phase thereof the implant of claim 1 or claim 2.
- 20 7. The implant of claim 1 or claim 2 comprising hollow fibers.
8. The implant of claim 2 having a pore volume between about 50% and about 80%.

- 5 9. A method of making a fiber-reinforced, porous, tissue scaffold implant
 material comprising fibers aligned predominantly in one direction,
 comprising:
- (a) dissolving a polymer in a suitable organic solvent to form a solution;
- 10 (b) dispersing the fibers in a suitable non-solvent for the polymer to
 form a suspension;
- (c) precipitating the polymer mixed with fibers as a coherent mass from
 solution by mixing the suspension and solution;
- (d) kneading and rolling the coherent mass of fibers and polymer to
 orient the fibers predominantly parallel to each other; and
- 15 (e) applying heat and vacuum pressure to the mass to foam and cure it.
10. A method for making a non-porous, fiber-reinforced implant material
 comprising compression molding of the porous material of claim 9 into a
 non-porous material.

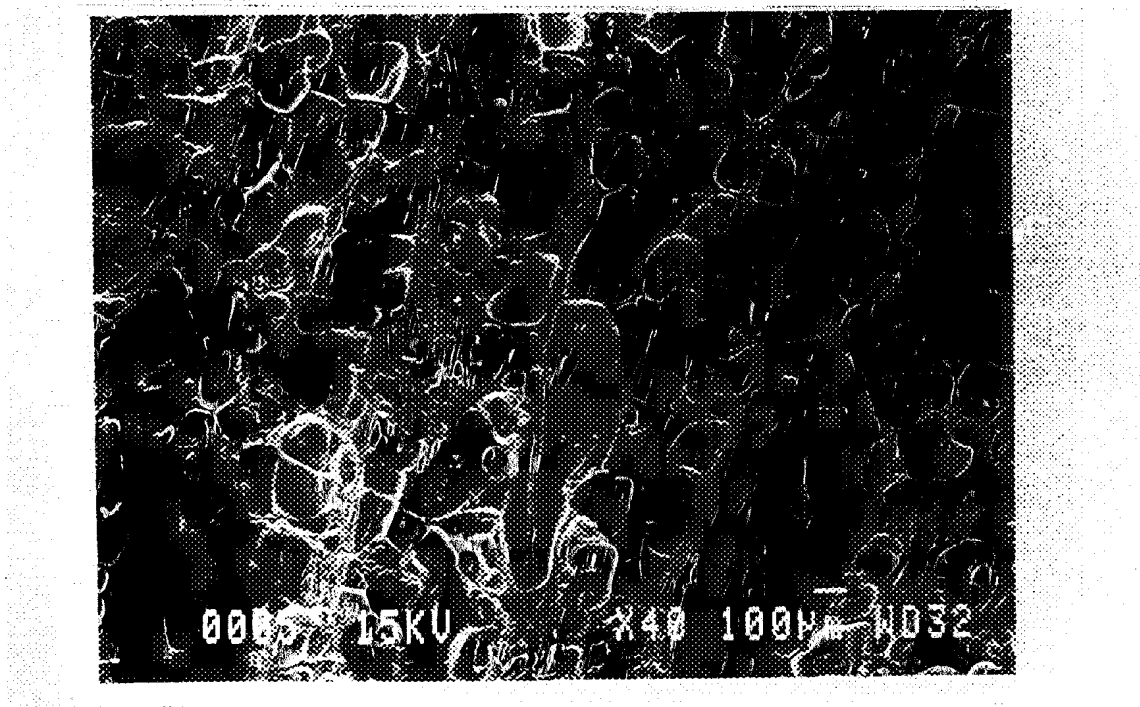


FIG. 1

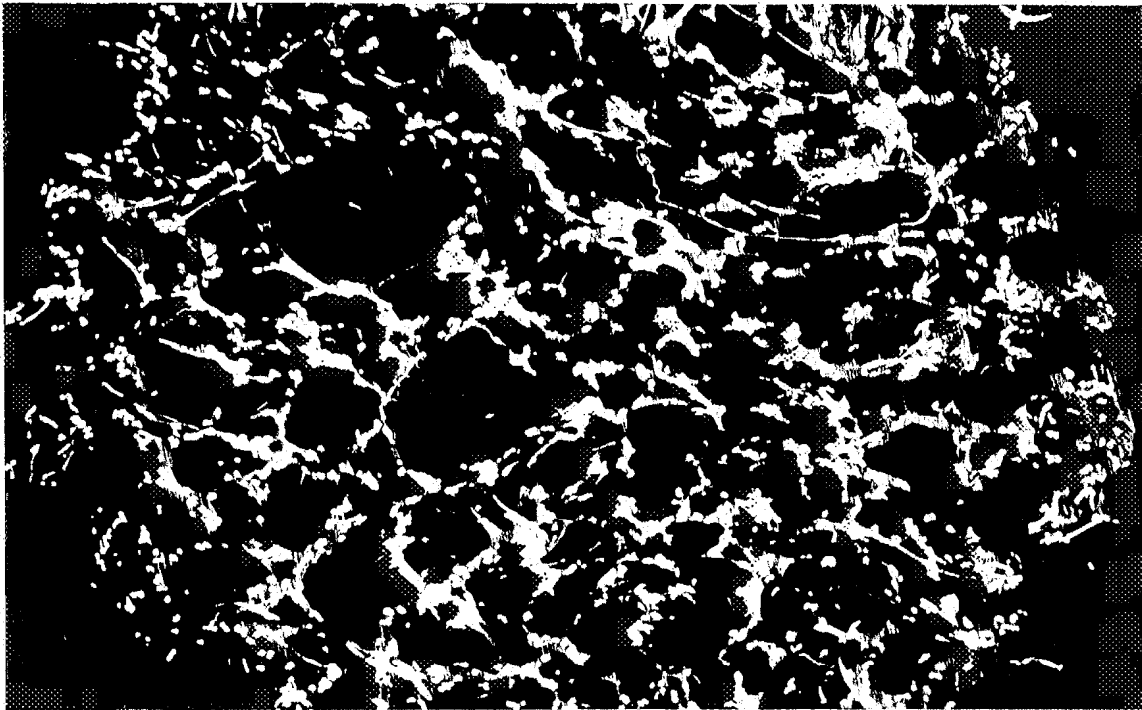


FIG. 2A

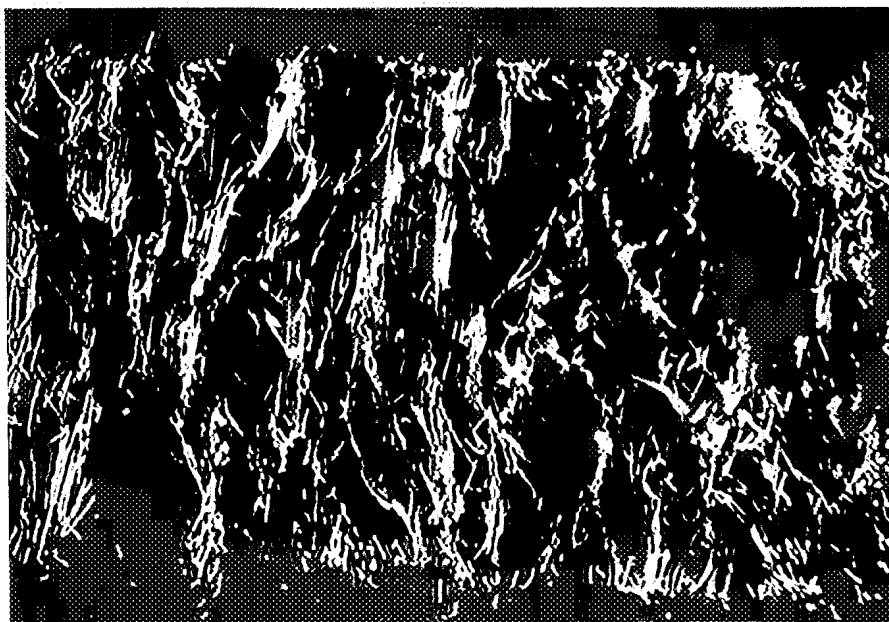
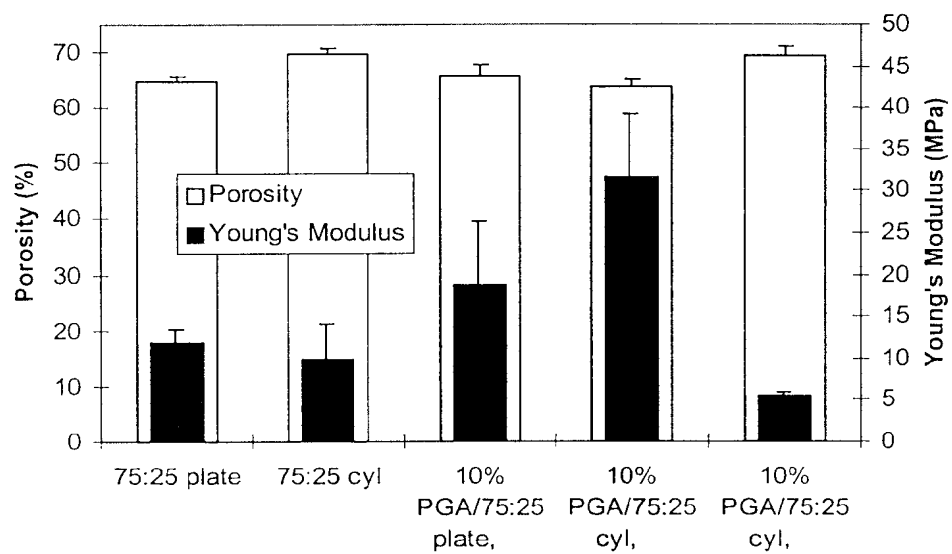
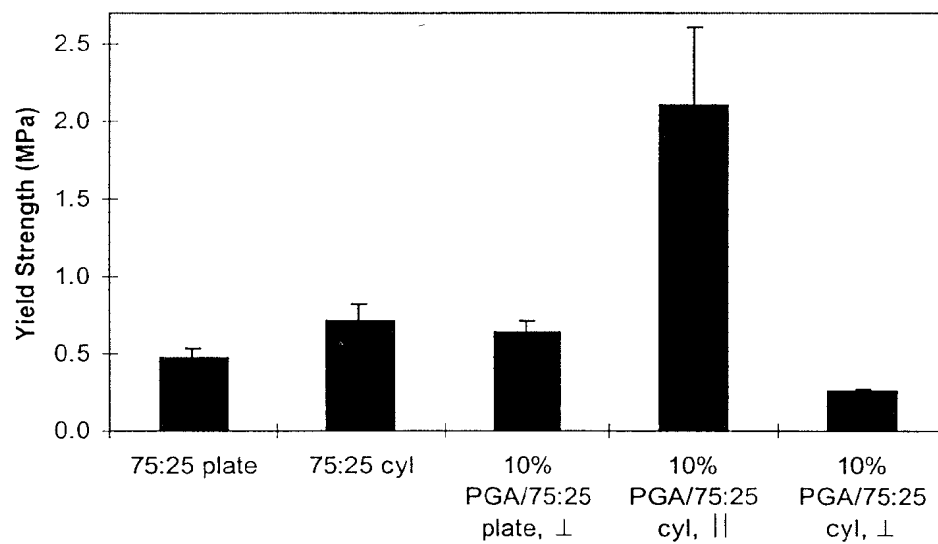
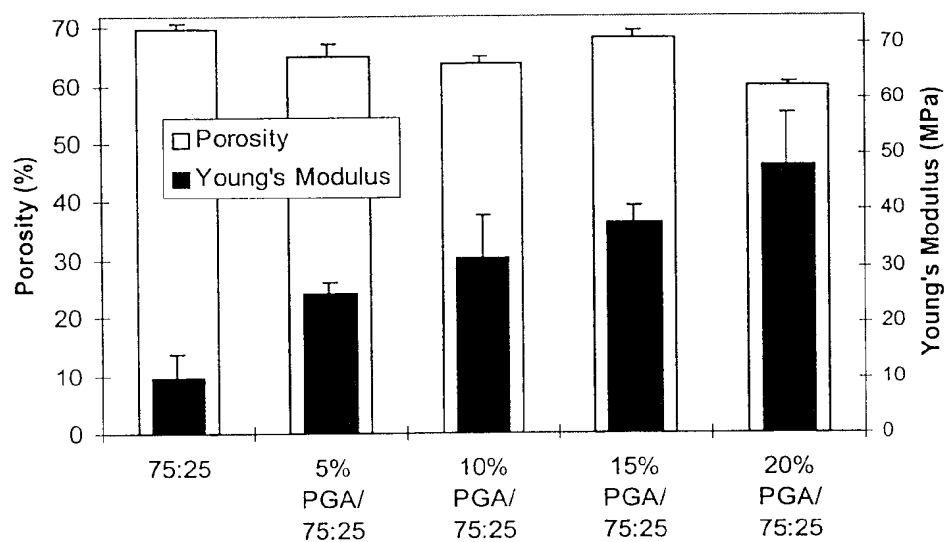
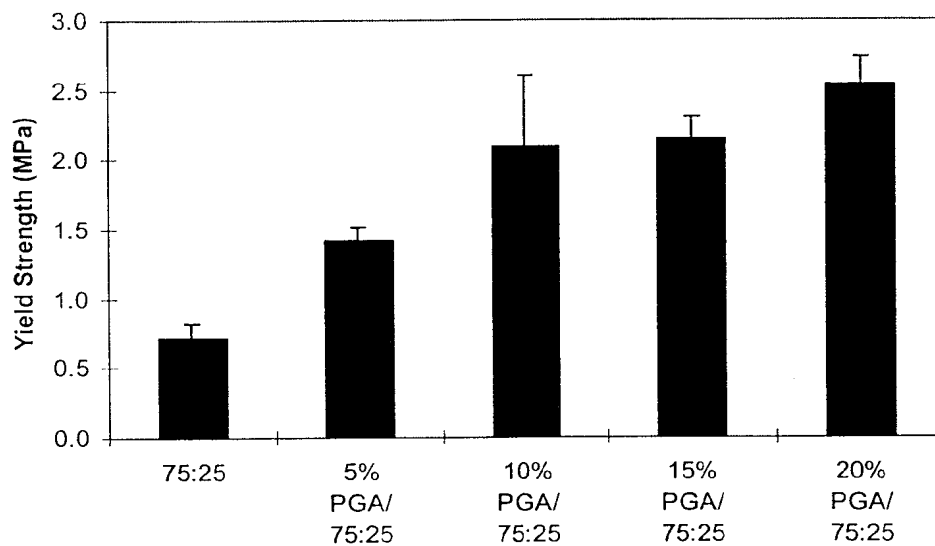


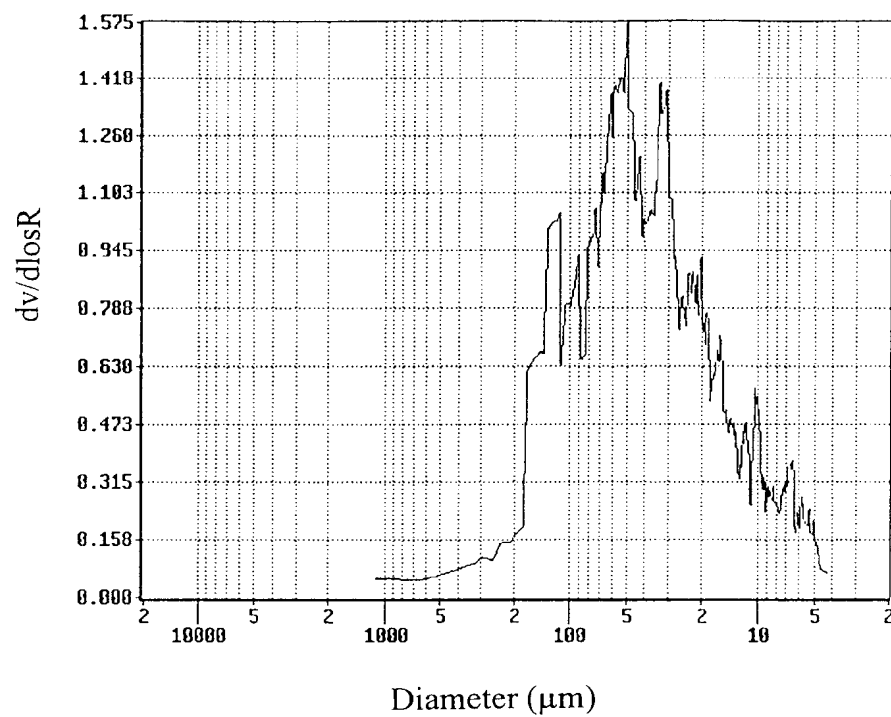
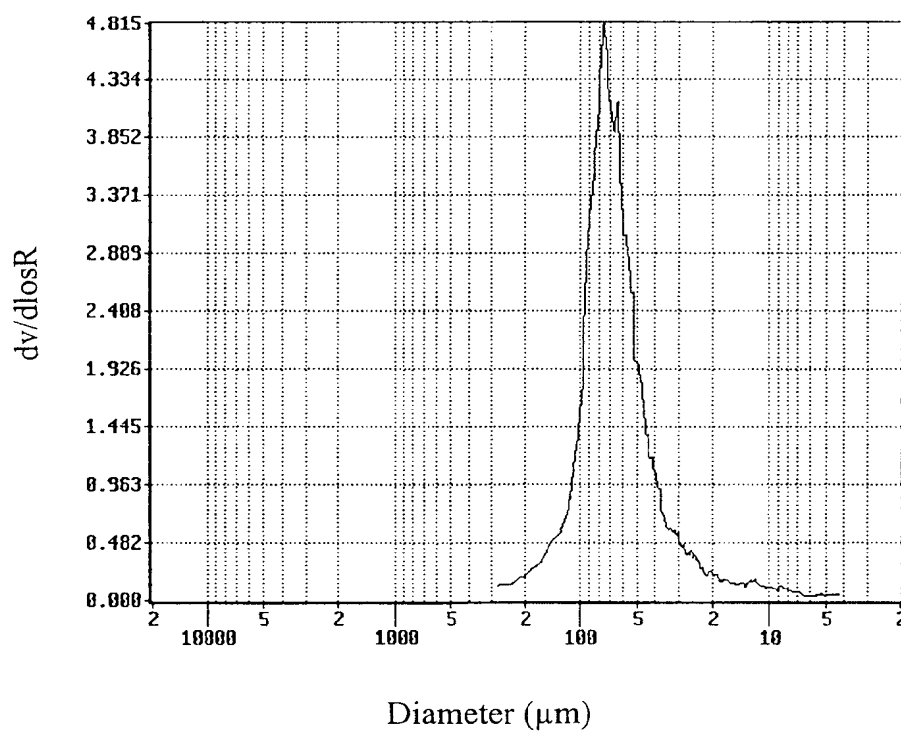
FIG. 2B

3/5

**FIG. 3****FIG. 4**

4/5

**FIG. 5****FIG. 6**

**FIG. 7A****FIG. 7B**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11007

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61F 2/30
US CL : 623/11, 16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 623/11, 16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,609,638 A (PRICE ET AL) 11 MARCH 1997, ENTIRE DOCUMENT.	1, 2
Y	US 3,662,405 A (BORTZ ET AL) 16 MAY 1972, ENTIRE DOCUMENT.	1, 2, 4, 6, 8
Y	US 5,607,474 A (ATHANASIOU ET AL), 04 MARCH 1997, ENTIRE DOCUMENT.	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

09 JULY 1998

Date of mailing of the international search report

30 JUL 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DAVID J ISABELLA

Telephone No. (703) 308-3060